**NHANES 2001–2002 Data Release**
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**Documentation for Laboratory Results**

# Laboratory 02 - Antibody to Hepatitis B Core Antigen, Antibody to Hepatitis B Surface Antigen, Hepatitis B Surface Antigen, and Antibody to Hepatitis C Virus (confirmed)

## (1) Documentation File Date – February 2006

## (2) Documentation File Name – Laboratory 02 - Hepatitis

## (3) Survey Years Included in this File Release – 2001–2002

## (4) Component Description

Hepatitis Viruses constitute a major public health problem because of the morbidity and mortality associated with the acute and chronic consequences of these infections. New immunization strategies have been developed to eliminate spread of hepatitis B and hepatitis A viruses in the United States. Recommendations have also been developed for the prevention and control of hepatitis C virus (HCV) infection Because of the high rate of asymptomatic infection with these viruses, information about the prevalence of these diseases is needed to monitor prevention efforts. By testing a nationally representative sample of the U.S. population, NHANES will provide the most reliable estimates of age-specific prevalence needed to evaluate the effectiveness of the strategies to prevent these infections. In addition, NHANES provides the means to better define the epidemiology of other hepatitis viruses including hepatitis D and E. In NHANES testing for markers of infection with hepatitis viruses will be used to determine secular trends in infection rates across most age and racial/ethnic groups, and will provide a national picture of the epidemiologic determinants of these infections.

## (5) Sample Description

### 5.1 Eligible Sample

**Antibody to hepatitis B core antigen (anti-HBc), antibody to Hepatitis B surface antigen (anti-HBs), and antibody to hepatitis C virus (anti-HCV confirmed)** Participants aged 6 years and older are eligible to be tested.

**Hepatitis B surface antigen (HBsAg), on anti-HBc positive samples** Participants aged 6 years and older are eligible to be tested.

**Antibody to Hepatitis B surface antigen (anti-HBs)** Participants aged two to five are tested.

## (6) Description of the Laboratory Methodology

### Hepatitis B Virus Core Antibody

ORTHO HBc ELISA Test System is a qualitative enzyme-linked immunosorbent assay for the detection of total antibody to hepatitis B virus core antigen (anti-HBc) in human serum or plasma. Anti-HBc appears in virtually all individuals infected with HBV and is an accurate serological marker of current and past infection.

Enzyme-linked immunosorbent assay (ELISA) procedures provide a means for routinely detecting antibodies to specific antigens. This FDA-licensed method is commercially obtained in kit form. The literature and instructions with each kit constitute the standard operating procedure (SOP) for the method.

### Hepatitis B Surface Antibody

The AUSAB EIA for anti-HBs uses the “sandwich principle” in a solid phase enzyme-linked immunoassay technique to detect antibody to HBsAg in serum or plasma. Anti-HBs appears after exposure to HBsAg and is a marker for immunity following infection or as a result of vaccination.

In the AUSAB EIA, patient speciment is incubated with polystyrene beads coated with human Hepatitis B Surface Antigen (HBsAg). Anti-HBs, if present binds to the solid phase antigen. After washing to remove unbound material, biotin tagged HBsAg (B-HBsAg) and rabbit anti-biotin, conjugated with horseradish peroxidase (anti-H-HRPO), are incubated with the beads to form antigen-antibody-antigen “sandwich” complexes. The presence of these complexes is indicated by a colorimetric reaction produced by the addition o-phenylenediamine (OPD), a substrate for HRPO. A yellow color develops in proportion to the amount of anti-HBs in the sample and is assessed by measuring absorbance at 492 nm using a spectrophotometer. The concentration of anti-HBs in the sample is assessed by comparison to absorbances of a panel of quantitative standards run in parallel with the AUSAB EIA testkit. Levels are expressed in milli-international units per mL (mum).

### Hepatitis B surface Antigen

The Auszyme monoclonal test is used to detect the presence of hepatitis B surface antigen (HBsAg), which indicates current infection with hepatitis B virus (HBV), Sensitive enzyme immunoassays used to detect the presence of HBsAg were first described by Engvall and Perlmann (1-3) and VanWeemen and Schuurs (4) in 1971. In 1976 and 1977, solid phase “sandwich” enzyme immunoassays for the detection of HBsAg were described by Wisdom (5), Wolters, et al,(6) and Wei, et al.(7). The production, characterization and application of monoclonal antibodies for the detection of HBsAg have previously been reported. (8-13)

Specimens nonreactive by the Auszyme Monoclonal tests are considered negative for HBsAg and are not be tested further. All specimens considered reactive initially are repeat tested in duplicate using the same procedure as that used in the initial test. If neither of the repeat tests is reactive, the specimen is considered negative for HBsAg. If the specimen is reactive in either of the repeat tests, the sample is considered repeatedly reactive.

**Hepatitis C Antibody Anti-HCV Screening ELISA**

Qualitative determination of the human antibody directed against hepatitis C virus (anti-HCV) in human serum or plasma is measured using direct solid-phase enzyme immunoassay. Results are expressed as "positive" or "negative" for anti-HCV. Positive specimens are repeated in duplicate according to the same procedure. Repeatedly positive specimens are tested supplementally using the RIBA Processor System (Chiron Corporation, Inc.). While the Chiron RIBA 3.0 Strip Immunoblot Assay (Chiron Corporation, Inc.).

**The Chiron RIBA HCV 3.0 Strip (confirmation test)**

Immunoblot Assay (SIA) is an in vitro qualitative enzyme immunoassay for the detection of antibody to hepatitis C virus (anti-HCV) in human serum or plasma.

Detection of anti-HCV by SIA methodology is based upon traditional Western and dot blotting techniques, in which specific immunogens (i.e. antigenic polyproteins) encoded by the HCV genome are immobilized onto a membrane support. Visualization of anti-HCV reactivity in specimens to the individual HCV-encoded proteins is accomplished using anti-human IgG enzyme-conjugates in conjunction with a colorimetric enzyme substrate. Qualitative determination of the human antibody directed against hepatitis C virus (anti-HCV) in human serum or plasma is measured using direct solid-phase enzyme immunoassay.

## (7) Laboratory Quality Control and Monitoring

The NHANES quality control and quality assurance protocols (QA/QC) meet the 1988 Clinical Laboratory Improvement Act mandates. Detailed quality control and quality assurance instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed QA/QC protocols.

## (8) Data Processing and Editing

Blood specimens are processed, stored and shipped to the Division of Viral Hepatitis, National Center for Infectious Diseases, National Centers for Disease Control and Prevention. Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed data processing and editing protocols. The analytical methods are described in the Analytic methodology section.

## (9) Data Access

All data are publicly available.

## (10) Analytic Notes for Data Users

The analysis of NHANES 2001-2002 laboratory data must be conducted with the key survey design and basic demographic variables. The NHANES 2001-2002 Household Questionnaire Data Files contain demographic data, health indicators, and other related information collected during household interviews. They also contain all survey design variables and sample weights for these age groups. The phlebotomy file includes auxiliary information such as the conditions precluding venipuncture. The household questionnaire and phlebotomy files may be linked to the laboratory data file using the unique survey participant identifier SEQN.

The age ranges and constraints for hepatitis testing are as follows:

- **Hep B-**The hepatitis B core antibody test is performed on all examinees 6 years old and older while the hepatitis B surface antibody test is performed on all examinees 2 years old and older. The surface antigen is tested only when the core antibody test is positive. The surface antigen test result is coded as negative when BOTH core antibody and surface antigen tests are negative.

**Note: Hepatitis B surface antigen will be released April 2006. Hepatitis B core antibody and surface antibody will be in a later release.**

- **Hep C-**The screening hepatitis C antibody test is performed on all examinees 6 years old and older. Samples testing positive for anti-HCV by the screening EIA test were tested in the confirmatory RIBA assay for antibody to Hepatitis C virus. Samples where the RIBA result was positive are reported as confirmed positive for antibody to HCV. Samples where the RIBA result was negative are reported as negative for antibody to HCV and indeterminate results are reported as indeterminate.

Samples that tested negative by the screening EIA test were not tested by RIBA. These samples were reported as negative for antibody to HCV. If the antibody to Hepatitis C virus was negative, weakly positive, weakly negative or indeterminate then the confirmatory assay to the antibody to Hepatitis C virus was reported as negative and included with the negatives from the screening assay.

## (11) References

1. N/A